

screening or detection of compounds which affect particular biological functions. The methods of the invention utilize fluorescent biosensor molecules which, when acted on by a compound of interest, cause an alteration in the cellular distribution of at least the fluorescent moiety. In one embodiment, the biosensors comprise heat shock proteins (hsp) fused to a fluorescent protein (e.g. jellyfish green fluorescent protein (GFP), or derivatives thereof). Such biosensors are located in the cytoplasm, but on stress activation translocate to the nucleus. In another embodiment biosensor proteins can be used to detect protease activity. Such protease biosensor proteins comprise one or more fluorescent proteins, a recognition signal which is cleaved by the protease, and at least one cellular localisation signal. The latter two components may be components of a single protein which is acted upon by the protease, or may be from heterologous sources. Due to the localisation signal, the biosensor protein is localised to a particular region of the cell once acted on by the protease of interest. The fluorescent protein is cleaved from the localisation sequence, and is free to migrate to other locations within the cell. The presence of a second localisation signal attached to the fluorescent protein enables the fluorescent protein to be directed to a different cellular compartment after cleavage of the protease recognition sequence. The change in distribution of the fluorescent protein can be detected using imaging methods with a high degree of spatial resolution. The methods and biosensors of the invention can be used to investigate a wide range of cellular activities and to screen compounds which modulate these activities. Biosensors containing a recognition site for caspase, for example, may be used for the screening of compounds which modulate apoptosis, while biosensors containing other protease recognition sites may be used for the detection of proteolytic toxins (such as anthrax lethal factor). The method provides improved target validation and candidate compound optimisation by combining many cell screening formats with fluorescence-based molecular reagents and computer based image evaluation, data analysis and automation, resulting in increased quantity and speed of data collection and faster evaluation of drug candidates. Sequences AA694372, A93376, represent fluorescent proteins (AA622881, B22885) which may be used as components of biosensor fusion proteins of the invention.

XX Sequence 717 BP: 170 A: 243 G: 203 G: 101 T: 0 other:

Alignment Scores:

Prod. No.:	Length:	717
Score:	1,250-140	217
Percent Similarity:	100.00%	2
Best Local Similarity:	99.1%	Mismatches: 0
Query Match:	99.2%	Indels: 0
DB:	21	Gaps: 0

US-09-866-538-6 (1-239) x AA694375 (1-717)

QY 1 MetValSerIysGlyGluGlnGluProHisGlyValValProIleValGlnLeuAsp 20
 DB 1 AFGHIGAGFAAGGAG 50
 QY 21 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 40
 DB 61 GGGDAGCTAAAGGAG 120
 QY 41 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 50
 DB 121 GGGDAGCTAAAGGAG 180
 QY 61 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 80
 DB 181 GGGDAGCTAAAGGAG 240
 QY 81 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 100
 DB 241 GGGDAGCTAAAGGAG 300
 QY 101 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 120
 DB 301 GGGDAGCTAAAGGAG 360

QY 121 ValAsnGlnIleLeuGluGlnGlnGlnGlnGlnGlnGlnGlnGlnGlnGlnGln 140
 DB 361 GGGDAGCTAAAGGAG 420
 QY 141 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 160
 DB 421 GGGDAGCTAAAGGAG 480
 QY 161 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 180
 DB 481 GGGDAGCTAAAGGAG 540
 QY 181 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 200
 DB 541 GGGDAGCTAAAGGAG 600
 QY 201 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 220
 DB 601 GGGDAGCTAAAGGAG 660
 QY 221 GIVASVVALASnGlyHisAAPHeserValSerGlyGluGlnGlnGlnGlnGlnGln 239
 DB 661 GGGDAGCTAAAGGAG 717

RESULT 7
 AA627575
 10 AA627575 standard, DNA, 717 BP.

AC AA627575:

TT 20-AMV-2000 (first entry)

DE DNA encoding RCFP signal domain.

KW Protease, biosensor, GFP, signal peptide, cell screening

KW assay, analysis, drug discovery, ss.

OS Unidentified.

PN W0200026408-A2.

XX 11-MAY-2000.

XX 29-OCT-1999; 99WO-0525431.

XX 40-OCT-1998; 98US-0106308.

XX 26-MAY-1999; 99US-0136078.

XX (CELL-) CELLIMICS INC.

XX Gulliano KA, Bright G, Olson K, Burroughs-Jennya S:

XX WPI: 2000-365644/31.

XX P-FSDB: AA679586.

XX Recombinant nucleic acid encoding a protease biosensor useful for

XX fluorescence based cell and molecular biochemical assays for drug

XX discovery comprising three operably linked nucleic acid sequences

XX Claim 5: Fig 29A: 218bp; English.

XX The present sequence is that of RNA encoding the GFP signal domain
 XX (see AA679586). The DNA can be used in a claimed recombinant nucleic
 XX acid encoding a protease biosensor. The nucleic acid (see AA679586-43)
 XX comprises: (1) a sequence (see AA627568-76) encoding at least 1
 XX detectable polypeptide signal, such as GFP; (2) a sequence (see
 XX AA627577-611) that encodes at least 1 protease recognition site; and
 XX (3) a sequence (see AA627611-26) that encodes at least 1 variant
 XX target sequence. An expression vector, a genetically engineered
 XX host cell and a recombinant protease biosensor are also claimed.
 XX A claimed method for identifying compounds that modify protease
 XX activity in a cell involves contacting a host cell that possesses

[illegible][illegible]

[illegible]

DB	Accession	Protein Name	Length	Accession	Protein Name	Length
52	201	Protein	768	201	Protein	768
53	661	Protein	768	661	Protein	768
54	661	Protein	768	661	Protein	768
55	661	Protein	768	661	Protein	768
56	661	Protein	768	661	Protein	768
57	661	Protein	768	661	Protein	768
58	661	Protein	768	661	Protein	768
59	661	Protein	768	661	Protein	768
60	661	Protein	768	661	Protein	768
61	661	Protein	768	661	Protein	768
62	661	Protein	768	661	Protein	768
63	661	Protein	768	661	Protein	768
64	661	Protein	768	661	Protein	768
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70	661	Protein	768	661	Protein	768
71	661	Protein	768	661	Protein	768
72	661	Protein	768	661	Protein	768
73	661	Protein	768	661	Protein	768
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75	661	Protein	768	661	Protein	768
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77	661	Protein	768	661	Protein	768
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96	661	Protein	768	661	Protein	768
97	661	Protein	768	661	Protein	768
98	661	Protein	768	661	Protein	768
99	661	Protein	768	661	Protein	768
100	661	Protein	768	661	Protein	768

[illegible][illegible]

[illegible][illegible]

[illegible][illegible]

[illegible]
$$\begin{aligned} \text{Euler's } \Gamma \text{ function: } \Gamma(x) &= \int_0^\infty t^{x-1} e^{-t} dt, \quad x > 0 \\ \text{Gamma function: } \Gamma(x) &= \frac{\pi}{\sin(\pi x)}, \quad x \notin \mathbb{Z} \end{aligned}$$



10

100


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1 ORGANISM: Acetobacter vinetianus
2
3 FEATURE:
4
5 NAME/KEY: misc_feature
6
7 LOCATION: (0) .. (0)
8
9 OTHER INFORMATION: EPRP
10
11 DB: 0.172.004.11

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Atmospheric conditions:	Pressure, MPa	1.430 ± 0.1	Length, m	2.70 ± 0.05
Temperature, °C	124.2 ± 0.0	Material	Carbon steel	2.45
Electrode diameter, mm	66.7 ± 4.4	Electrode voltage, V	5	2.45
Electrode material	7.7 ± 0.9%	Material thickness, mm	4	2.45
Electrode diameter, mm	9.7 ± 0.8	Insulation	100 ± 5	2.45
Geometry	4	Capacitance	0	2.45

$$11.5 \pm 0.4 \text{ MeV}, \quad 1.38 \pm 0.1 \text{ MeV} \times 1.55 \pm 0.09 \pm 1.2 \text{ MeV}, \quad 1.1 \pm 0.2 \text{ MeV}.$$
[illegible]

RECEIVED 11
 035 009 816 010 12
 2. Specimen No. 12. Applicant code 052/009 816 010
 2. Patent No. 64,919-4
 2. GENERAL INFORMATION
 2. Applicant: BARRY, ROBERT Y.
 2. Applicant: INVENTION: FLOW-OUT PEN PROBLEM
 2. INVENTOR: FLOW-OUT PEN PROBLEM

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1 FILEREFERENCE: 07572707.0001
2 CURRENT APPLICATION NUMBER: 000000410, 919
3 CURRENT FILING DATE: 1999-09-21
4 NUMBER OF SEQ ID NOS: 64
5 SOFTWARE: FASTSEQ for Windows Version 4.0
6 SEQ ID NO: 12
7 LENGTH: 720
8 TYPE: DNA
9 ORGANISM: Acinetobacter Victoria
10 DS-09 416, 919, 12

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Alignment Scores:		
Speed: No.:	1, 416, 151	7.70
Score:	1,262, 00	2.64
Percent Similitarity:	98, 74%	4
Percent Similitarity:	97, 45%	5
Percent Similitarity:	97, 10%	0
Query Match:	4	0
DB:		
US-09 806 648-6 (1, 2, 40) × US-09 416-919 1, 2 (1, 2, 70)		

$$15.7 \pm 1.2 \text{ (1.4-19.1)} \times 10^{-19} \text{ s}^{-1} \times (1.5-2.1) \times 10^{-6} \text{ s} \times 10^{-9} \text{ m}^3 \text{ mol}^{-1}$$
[illegible]

RESULT 12
US-09-062-102 Z
Sequence Z, Application US/09062102
; Patent No. 6,403,14

ALPHEANT; KASHER [Fr., MAISON]M.

[illegible]

[illegible][illegible]

[illegible]

DB	61	000
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US-09-866-538-6 (1-239) x US-09-866-538-6 (1-720)

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QY 1 MGVVLSRTYSGYGLVGLILEPPECTHGLYVALVLPGLILEGVAIGLILGLASP 20
DB 1 AIGGTAAGAGAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 60
QY 21 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 40
DB 41 GGGGAGCTAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 120
QY 41 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 60
DB 121 GGGGAGCTAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 180
QY 61 LENVALTHTLTHLSTHTPLSLYVALISPGYGLVGLILEGVAIGLILGLASP 80
DB 181 CTGGTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 240
QY 81 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 100
DB 241 GAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 300
QY 101 PHELYASVSPSLYSGLYSLYSLYSLYSLYSLYSLYSLYSLYSLYSLYSLY 120
DB 301 TTCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 360
QY 121 VALASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 140
DB 361 GTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 420
QY 141 LYSGLILEGVAIGLILGLASP 160
DB 421 AAGCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 480
QY 161 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 180
DB 481 GTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 540
QY 181 ASPISTYGLINGLISARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 200
DB 541 GAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 600
QY 201 TYTLCUGGTHGLINSERVALISPGYGLVGLILEGVAIGLILGLASP 220
DB 601 TACCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 660
QY 221 LENVALTHTLTHLSTHTPLSLYVALISPGYGLVGLILEGVAIGLILGLASP 240
DB 661 CTGGTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 720

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RESULT 9

US-09-920-922-1
 Sequence 1, Application US/09920922
 Patent No. US2002008488A1
 GENERAL INFORMATION:
 APPLICANT: MIYAWAKI, ATSUSHI
 APPLICANT: SAKANO, ASAKO
 TITLE OF INVENTION: METHOD FOR METABOLISM
 FILE REFERENCE: 11284-012801
 CURRENT APPLICATION NUMBER: US 09/920,922
 PRIOR FILING DATE: 2001-08-02
 PRIOR APPLICATION NUMBER: JP 2000-217166
 NUMBER OF SEQ ID NOS: 9
 SOFTWARE: FASTSP for Windows Version 4.0
 SEQ ID NO: 1
 LENGTH: 720
 TYPE: DNA
 ORGANISM: A. thaliana
 FEATURE:
 NAME/FEATURE: CDS
 LOCATION: (1)...(717)

US-09-920-922-1

Alignment Scores:
 Ident. No.: 2,836,128 Length: 720
 Score: 1242.00 Matches: 233
 Percent Similarity: 98.74% Conserved: 4
 Best Local Similarity: 97.49% Mismatches: 4
 Query Match: 97.18% Indels: 0
 DB: 10 Gaps: 0

US-09-866-538-6 (1-239) x US-09-920-922-1 (1-720)

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QY 1 MGVVLSRTYSGYGLVGLILEPPECTHGLYVALVLPGLILEGVAIGLILGLASP 20
DB 1 AIGGTAAGAGAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 60
QY 21 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 40
DB 61 GGGGAGCTAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 120
QY 41 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 60
DB 121 GGGGAGCTAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 180
QY 61 LENVALTHTLTHLSTHTPLSLYVALISPGYGLVGLILEGVAIGLILGLASP 80
DB 181 CTGGTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 240
QY 81 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 100
DB 241 GAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 300
QY 101 PHELYASVSPSLYSGLYSLYSLYSLYSLYSLYSLYSLYSLYSLYSLYSLY 120
DB 301 TTCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 360
QY 121 VALASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 140
DB 361 GTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 420
QY 141 LYSGLILEGVAIGLILGLASP 160
DB 421 AAGCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 480
QY 161 GIVASVVAASGTHLSARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 180
DB 481 GTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 540
QY 181 ASPISTYGLINGLISARFHESEVVALISPGYGLVGLILEGVAIGLILGLASP 200
DB 541 GAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 600
QY 201 TYTLCUGGTHGLINSERVALISPGYGLVGLILEGVAIGLILGLASP 220
DB 601 TACCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 660
QY 221 LENVALTHTLTHLSTHTPLSLYVALISPGYGLVGLILEGVAIGLILGLASP 240
DB 661 CTGGTGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 720

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RESULT 10

US-09-554-000-1
 Sequence 1, Application US/09540000
 Patent No. US2002016536A1
 GENERAL INFORMATION:
 APPLICANT: MIYAWAKI, ATSUSHI
 APPLICANT: USION, ROGER Y.
 TITLE OF INVENTION: FLUORESCENT PROTEIN SENSORS FOR
 DETECTION OF ANALYTES
 FILE REFERENCE: 02257-042001
 CURRENT APPLICATION NUMBER: US 09/554,000
 PRIOR FILING DATE: 2000-04-20
 PRIOR APPLICATION NUMBER: 09/518,252

[illegible][illegible]

